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Absorption of Lead from Drinking Water with
Varying Mineral Content

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CHAPTER XXV

ABSORPTION OF LEAD FROM DRINKING WATER WITH VARYING MINERAL CONTENT

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ABSTRACT

Lead (Pb) (200 ppm) was administered via drinking water to rats for nine weeks. In addition, the rats were grouped so that they received 75, 100, 150 and 250% of the minimum daily requirements (MDR) of calcium (Ca), iron (Fe), and magnesium (Mg) as required for normal growth. The exposures were arranged so that no more than one element was varied within the same animal groups, while maintaining 100% of the MDR of all other elements and nutrients.

Blood lead analyses were performed at 0, 1, 2, 3, 7, 8, and 9 weeks after exposure. Food and water consumptions and body weights were measured each week (0-9).

Some gross differences were seen both within and among the groups with respect to milliliters water consumed vs. body weight and grams of food consumed vs. body weight. During the first few weeks of exposure, the mean blood lead in animals fed 75% MDR of Ca, Fe, and Mg appeared less than those receiving 100% MDR of the corresponding elements. There was a decrease in mean blood lead concentration in animals receiving 150 and 250% of the MDR of Fe at the 7-9 week time period; however, this was probably due to a slight decrease in water consumption during this time period. There seemed to be no appreciable differences in mean blood lead concentrations of groups exposed to high concentrations of Ca and Mg. However, analysis of the data after correcting for varying Pb dose points to the conclusion that dietary magnesium has no effect on uptake of lead whereas dietary iron decreases blood lead concentration and calcium only increases blood lead concentration after extended exposure (7-9 weeks).

TABLE I.
Elemental Content of Experimental Rat Diet.

Element	Mg/Kg (ppm)
Calcium	4500
Cooper	5.6
Iron	20
Magnesium	300
Manganese	60
Potassium	2000
Sodium	600
Zinc	13.3
Sulfur	Trace (MDR as per vitamins)
Iodide	.17
Chloride	600
Phosphorous	4400

INTRODUCTION

The possible physiological changes arising from exposure to lead in our environment depend upon the amount of the metal ingested and absorbed and/or retained. Retention of lead in animals varies according to species, ages within species, and nature of the diet. There are some essential dietary components which may have an influence on the retention of lead (Baltrop et al., 1975; Petering, 1980). Calcium was one of the first dietary compounds to be implicated as having a metabolic relationship with lead absorption. An early clinical study was performed by Aub (1953), whereby lead intoxicated human subjects were given high levels of calcium to reduce blood lead levels. However, recent studies by Quarterman et al. (1977) indicate that an increase of dietary calcium to a lead intoxicated rat reduces the actual rate of lead loss from the body rather

TABLE II.
Design of Experimental Animal Groups with
Respect to Supplement Diets with Pb, Ca, Mg and Fe

<u>Low Lead Control (.06 ppm)</u>				
<u>Group I</u>				
MDR-Fe				
MDR-Ca				
MDR-Mg				
<u>200 ppm Lead in Drinking Water</u>				
<u>Group II</u>	<u>Group III</u>	<u>Group IV</u>	<u>Group V</u>	<u>Group VI</u>
MDR-Fe	75% MDR-Fe	MDR-Fe	MDR-Fe	150% MDR-Fe
MDR-Ca	MDR-Ca	75% MDR-Ca	MDR-Ca	MDR-Ca
MDR-Mg	MDR-Mg	MDR-Mg	75% MDR-Mg	MDR-Mg
<u>Group VII</u>	<u>Group VIII</u>	<u>Group IX</u>	<u>Group X</u>	<u>Group XI</u>
MDR-Fe	MDR-Fe	250% MDR-Fe	MDR-Fe	MDR-Fe
150% MDR-Ca	MDR-Ca	MDR-Ca	250% MDR-Ca	MDR-Ca
MDR-Mg	150% MDR-Mg	MDR-Mg	MDR-Mg	250% MDR-Mg
MDR = Minimum Daily Requirement for normal growth				
MDR-Calcium = 6000 ppm				
MDR-Iron = 25 ppm				
MDR-Magnesium = 400 ppm				

than increasing lead loss. Other literature suggests that lead affects calcium metabolism. One study by Six et al. (1970) indicates that rats given lead in their drinking water had small decreases in serum calcium levels regardless of their levels of dietary calcium. The study also showed that adding lead to a low calcium diet greatly increased kidney calcium and lead content.

Previous studies by Six et al. (1972) and Barton et al. (1978) have indicated that feeding of an iron deficient diet demonstrated an increased retention of lead in the whole body.

Iron, magnesium and calcium are the major elements of hard water and all have been reported to be readily absorbed in the small intestine and therefore may compete with lead for some common transport system or metabolic interactions as explained by Conrad et al. (1978) and Mahaffey et al. (1980). The present work was undertaken to determine the effects upon lead absorption by increasing the dietary concentration (above normal growth requirements) of those major ions (iron, magnesium and calcium) which constitute water hardness.

TABLE III.
Body Weight, Food and Water Consumption Data
Summary Data Point Week 1-3

Diet	Body Wt. (Gm)	Water (Ml)	Food (Gm)	Ml Water	Gm Food
				100 Gm Body Wt. Per Week	100 Gm Body Wt. Per Week
75% MDR-Ca	352	175	144	49.7	40.9
100% MDR-Ca	341	167	141	49.6	41.6
150% MDR-Ca	309	149	140	48.9	46.2
250% MDR-Ca	284	148	116	51.8	40.8
75% MDR-Fe	363	178	145	48.6	40.2
100% MDR-Fe	341	167	141	49.6	41.6
150% MDR-Fe	345	184	152	54.9	44.5
250% MDR-Fe	352	148	151	42.0	42.9
75% MDR-Mg	351	149	140	42.5	40.1
100% MDR-Mg	341	167	141	49.6	41.6
150% MDR-Mg	333	153	148	46.8	45.5
250% MDR-Mg	349	158	150	45.2	43.0
No Lead Control	344	189	150	54.9	43.6

METHODS

Diet. Pelleted rat chow was used containing 100% of the minimum daily requirements (MDR) of nutrients and minerals necessary for normal growth and development as recommended by the National Academy of Science (1966) with the exception of calcium, iron and magnesium. These three elements were contained in the chow at a concentration of 75% of MDR. The elemental salt concentration of the rat chow used in this experiment and confirmed by Inductively Coupled Plasma Spectroscopy analysis is found in Table I.

Calcium, iron, and magnesium were added to deionized distilled drinking water at concentrations (assuming a normal water intake) that would provide for a total intake of these elements at 1.0, 1.5, and 2.5 times the MDR for normal growth and development (MDR 25 Mg/Kg for iron, 6000 Mg/Kg for Ca, 400 Mg/Kg for Mg). Lead at 200 ppm was administered via drinking water to all experimental animals except the no lead control group. This Pb dosage has been shown by Six et al. (1970) to be the maximum dose of Pb fed for a ten week period that does not produce significant alterations in hematopoiesis, renal size, histology, and renal function in the rat. The chemical forms of the elements used were $MgCl_2 \cdot 6H_2O$, $CaCl_2 \cdot 2H_2O$, $FeCl_3$ and $PbCl_2$ chloride salts.

Experimental Design. One hundred and ten male albino Sprague-Dawley rats weighing 200-225 grams were divided into eleven groups, ten rats per group, and fed a purified rat diet to contain 75% MDR of the elements Ca, Fe, and Mg supplemented by appropriate amounts in the drinking water to give intakes of the elements indicated in Table II.

TABLE IV.
Body Weight, Food and Water Consumption Data
Summary Data Point Week 7-9

Diet	Body Wt. (Gm)	Water (Ml)	Food (Gm)	Ml Water	Gm Food
				100 Gm Body Wt. Per Week	100 Gm Body Wt. Per Week
75% MDR-Ca	487	178	158	36.9	32.4
100% MDR-Ca	480	186	153	39.2	31.8
150% MDR-Ca	453	149	146	32.9	32.2
250% MDR-Ca	420	132	157	31.6	37.5
75% MDR-Fe	502	192	162	38.4	32.3
100% MDR-Fe	480	186	153	39.2	31.8
150% MDR-Fe	508	169	170	33.4	33.4
250% MDR-Fe	493	161	160	32.6	32.4
75% MDR-Mg	503	173	156	34.6	30.9
100% MDR-Mg	480	186	153	39.2	31.8
150% MDR-Mg	471	197	157	41.9	33.3
250% MDR-Mg	485	192	158	40.7	33.0
No Lead Control	480	210	142	43.7	29.6

The animals were housed individually in stainless steel cages for the nine week experimental period. The body weight, food and water consumption for each animal was recorded weekly. Blood samples were collected with heparinized capillary tubes for all animals at the end of 0, 1, 2, 3, 7, 8, and 9 weeks by retro orbital sinus puncture. After collection, each blood sample was immediately transferred into a tube containing Metexchange M reagent of Environmental Science Associates (1982). Lead analyses were performed within 24 hours of collection.

Analytical Methods. Blood lead analyses were performed by the method of anodic stripping voltammetry as outlined by Environmental Science Associates (1978). This method requires only 200 μ l of whole blood, making it possible to analyze serial samples from the same animal over a period of exposure time.

RESULTS

One week prior to the start of exposure, the baseline blood lead levels for all animals were determined to be $< 2 \mu\text{g}\%$. The no lead basal rat diet was analyzed to contain 0.06 ppm lead. These low baseline lead values indicate that any effects obtained could not be attributable to the basal diet or previous lead exposure.

The mean body weights, food and water consumption data for weeks 1-3 and 7-9 of exposure are reported for each group of animals in Tables III and IV.

Since body weight is generally indicative of food and water consumption, the ratios of intake to body weight were calculated. The relative intake of water and food per unit body weight for all exposed groups decreased during the later weeks of the study.

TABLE V.
Effect of Dietary Calcium, Iron and Magnesium
on Blood Lead Absorption

Diet	Blood Lead $\mu\text{g}\%$ Week 1-3		Blood Lead $\mu\text{g}\%$ Week 7-9	
	Mean	Standard Error	Mean	Standard Error
75% MDA-Ca	53.0	3.3	48.2	1.5
100% MDR-Ca	61.8	3.8	49.8	3.3
150% MDR-Ca	48.5	2.9	45.8	2.3
250% MDR-Ca	51.2	2.7	56.4	5.4
75% MDR-Fe	56.9	4.4	53.9	3.9
100% MDR-Fe	61.8	3.8	49.8	3.3
150% MDR-Fe	54.6	3.1	44.0	2.0
250% MDR-Fe	48.1	4.1	39.4	2.6
75% MDR-Mg	53.3	2.9	48.2	3.0
100% MDR-Mg	61.8	3.8	49.8	3.3
150% MDR-Mg	58.3	3.9	56.6	2.7
250% MDR-Mg	59.2	5.6	52.7	7.9
No Lead Control	4.5		4.8	
100% MDR				

The no lead control group appeared to consume slightly more water per unit body weight than those exposed to lead and other experimental elements. This small difference may be attributed to the taste associated with iron and the other elements added to the experimental groups' water.

Table V consists of blood lead data for all experimental groups. Data from weeks 1, 2, and 3 were pooled with the mean blood lead values presented for each diet. Data points at week 7, 8, and 9 were similarly calculated.

Statistically, as would be expected, there was a highly significant difference in the mean blood lead levels of the no lead control group and all other lead exposed experimental groups for both 1-3 and 7-9 weeks time intervals.

In testing the effects of iron, calcium, and magnesium in the diet upon blood lead concentration (assuming an equal number of animals were exposed to each dietary regimen and assuming that lead intake was constant over the different dietary groups), the usual statistical approach would be analysis of variance followed by multiple comparison tests to identify differences in dietary regimens in terms of observed blood lead levels. This approach, however, was not satisfactory since large variations in consumption resulted in significant varia-

tions in the intake of dietary constituent intake and lead, both within and among the dietary groups.

Hence, a regression analysis approach was selected with each observation consisting of the blood lead and intake data for one animal during a week. Since food and water intake vary with body weight, weekly intakes were corrected for body weight, i.e., weekly intake for each animal was divided by the body weight of the respective animal. Reflecting the belief that the two observation periods (weeks 1-3 and 7-9) should be summarized separately, mean relative intakes for each period were used in the analysis. The form of the regression model for each period was as follows:

$$\text{Bld Pb} = b_0 + b_1 (\text{Ca}) + b_2 (\text{Mg}) + b_3 (\text{Fe}) + b_4 (\text{Pb})$$

where b_0 = intercept

and b_i , $i = 1 - 4$ are coefficients of the constituent and lead intakes

The results of the regression analysis of mean blood lead on the mean relative dietary intake (weekly) is shown in Table VI, indicating a statistical significance for iron at both time periods and for calcium at time period week 7-9.

CONCLUSIONS

When mean blood lead concentration data are observed as dietary groups there were no obvious statistical differences between the groups because of the variability of the data within each group. However, when each animal's data were statistically analyzed as an individual data point, there was some significance in that as the animals were exposed to increased iron in their diets their blood lead levels tended to decrease.

These blood lead data correspond to the lead absorption data findings of Barton et al. (1978), who determined that iron loading above MDR inhibited lead absorption. This could be explained in that iron and lead are absorbed in the duodenum according to Bivin et al. (1979) and may in fact compete for absorption sites in the intestinal mucosa.

TABLE VI.
Multiple Regression of Mean Blood Lead
on Mean Relative Dietary Intake

Parameter	Est.	Weeks 1-3		Est.	Weeks 7-9	
		t value	Prob >t		t value	Prob >t
Intercept	45.466	7.76	0.0001	12.590	2.04	0.045
Ca	-0.002	-1.86	0.067	0.006	3.34	0.001
Mg	0.027	1.30	0.198	0.017	0.77	0.443
Fe	-0.508	-2.10	0.039	-0.685	-2.41	0.019
Pb	0.177	3.54	0.0007	0.407	5.98	0.0001
Overall Model:	F value	Prob >F		F value	Prob >F	
	3.02	0.0072		16.20	0.0001	

In the case of calcium, blood lead levels in our study increased as the calcium intake increased but only at the 7-9 week period (extended exposure). This differs from the absorption study of Quarterman et al. (1978), as they reported an increase in dietary calcium (200% MDR) at the time of lead exposure would decrease the amount of lead absorbed. Kathryn Six et al. (1979) did demonstrate a 4 fold increase of blood lead with a diet containing much less calcium (15% MDR). Our study did not show any different blood lead levels between those animals receiving 75% and 100% MDR of calcium.

Magnesium seemed to have no effect on blood lead concentrations. This is explained perhaps because of the site of absorption in the small intestine (Bivin et al., 1979). Lead is absorbed primarily in the duodenum while magnesium is absorbed in the ileum; therefore, there is no competition for available mucosae cell sites.

Our study differed from other reported work in that a series of blood samples were taken, over time, from the same animal. Therefore, biological differences due to exposure times were somewhat more realistic although large variations of blood lead values existed for each exposure group.

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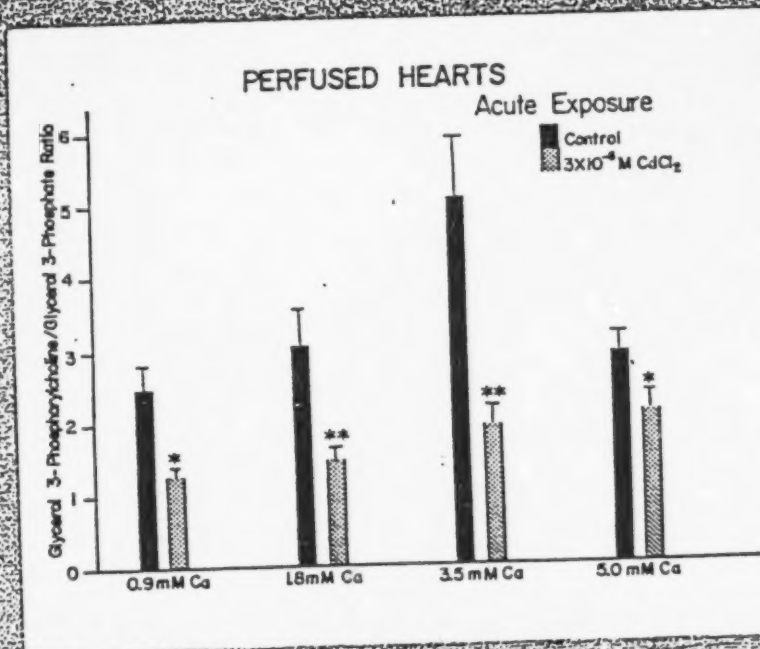
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